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REVIEW

Surface enhanced Raman spectroscopy and its application to molecular and cellular analysis

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Abstract In this paper, we review the state-of-the-art in surface-enhanced Raman scattering (SERS) based optical detection techniques with an application focus on cancer diagnostics. As we describe herein, SERS has several analytical, biological and engineering advantages over other methods including extremely high sensitivity, inherent molecular specificity of unlabeled targets, and narrow spectral bands. We review advances in both in vitro and in vivo applications of SERS and examine how technical issues with the technology are being addressed. A special technology focus is given to emerging optofluidic devices which aim to merge microfluidic and optical detection technologies into simple packages. We conclude with a brief discussion of some of the emerging challenges in the field and some of the approaches that are likely to enhance their application.

Keywords Optical biosensors · Surface enhanced Raman scattering · SERS · Cancer · Optofluidics · Tip-enhanced Raman spectroscopy

1 Introduction

Human cancer is a complex disease commonly induced by genetic instability and accumulation of multiple molecular alternations (Hanahan and Weinberg 2000; Verville and Sanderson 2000; Hahn and Weinberg 2002). As early stage diagnosis (in advance of the point where cancer cells have metastasized into other parts of the body) is relatively well correlated with improved survival rates, there has been extreme interest as of late in developing molecular detection technologies which can screen for circulating biomarkers or cellular events indicative of a cancer state. Most coarsely, biosensors and other methods of detecting the genetic, immunological or cell based markers associated with cancer can be separated by their transduction mechanism as either electrical, mechanical, and optical techniques (Huh et al. 2008). Electrical devices typically rely on detecting a change in surface charge or capacitance as a result of binding between a surface immobilized probe and a solution phase target. For example, Zheng et al. (2005) demonstrated the electrically multiplexed detection of cancer protein markers using arrays of silicon-nanowire based field-effect devices. The advantage of these devices are typically sensitivity of the technique and simplicity of the measurement (often only a conductance measurement is required) but they can be limited by interference with background conductivities and non-specific binding. Mechanical devices such as vibrating or deflecting cantilevers, piezoelectric membranes or surface acoustic waveguide devices have similarly been developed and applied to the detection of cancer markers. Such devices have also proven enormously successful, exhibiting extremely high sensitivities (Ilic et al. 2005; Arlett et al. 2006) but suffer from significant loss of performance in viscous fluids.

In spite of the advances in these areas, optical detection techniques still remain the most commonly used biosensing mechanism (Erickson et al. 2008; Fan et al. 2008). Of the numerous different implementations: surface enhanced Raman spectroscopy or SERS (Liu et al. 2007; Kneipp et al. 2005), surface plasmon resonance or SPR (Campagnolo

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et al. 2004; Huang et al. 2005), Fourier transform infrared spectroscopy or FT-IR (Riley et al. 2006; Hammody et al. 2007), and traditional fluorescent spectroscopy (Petrovsky et al. 2003) have been recently applied to cancer diagnostics. Fluorescence detection is likely the one most commonly used for bimolecular detection because of its high sensitivity and low detection limits for biologically relevant species. Though well developed, one disadvantage of this technique tends to be the added complication of labeling the target and the resulting potential for the label to interfere with the molecular interaction by blocking a binding site. Over the last decade, SPR has emerged as likely the preferred label-free optical method. This approach relies on detecting biding-induced refractive index variation at metal/dielectric interfaces by observing changes in an optimal plasmon coupling angle or wavelength. While very powerful, the technique itself relies on the relatively non-specific measure of mass accumulation as the probed quantity and thus any changes due to non-specifically bound molecules cannot be differentiated from the target.

To address some of these limitations, SERS optical sensing has recently attracted considerable attention for both in vitro and in vivo medical diagnostics (Qian et al. 2008). As we will discuss in greater detail below, interest in the approach is commonly related to the ability to provide label-free (in some cases) and molecularly specific information about the target of interest. In this review, our goal will be to provide a brief review of the SERS mechanism focusing on its advantages in molecular detection as related to cancer diagnostics. A number of recent reviews have been published on the use of SERS for detecting a broad range of different analytes (Sha et al. 2007; Lin et al. 2008; Qian and Nie 2008) and for more general information on SERS, readers are referred to those works. The first subsection below provides an overview and some of the history of SERS, followed by a general description of the different formats in which it has been implemented. Section 4 introduces recent implementations for SERS for molecular and cellular diagnostics. Sections 5 and 6 describe the natural extension of some of these techniques to single molecule and multiplexed analysis. Section 7 describes some of the techniques which have been developed for in vivo (or in situ) analysis. The final section provides a review of some of the novel "optofluidic" devices which have recently been demonstrated for SERS based in vitro diagnostics.

2 Brief overview of the surface enhanced Raman spectroscopy (SERS)

Raman spectroscopy has long been an important analytical method for the specific identification of molecules. Put simply, the technique involves shining a laser source onto an unknown chemical sample. Of the light that is absorbed by the sample, most of it will be scattered back at the same laser wavelength however a small portion of it will be inelastically scattered at series of different wavelengths that are indicative of the vibrational transitions in the molecule. Because different molecules have different vibrational modes, the spectrum of the inelastically scattered light can be thought of as analogous to a molecular fingerprint, uniquely identifying the interrogated molecule. Despite this high specificity, traditional Raman spectroscopy was considered limited because of the very poor efficiency of the inelastic scattering processes and thus the relatively weak signal.

This problem was addressed over 30 years ago with the advent of surface enhanced Raman spectroscopy or SERS. In SERS, the target molecule is brought into close proximity to a metallic (typically Ag, Au or Cu) surface with nanoscopically defined features or in solution next to a nanoparticle with a diameter much smaller than the wavelength of the excitation light. When light is incident on the surface or particle, a surface plasmon mode is excited which locally enhances the electromagnetic energy in the vicinity of the target molecule, significantly enhancing the intensity of the inelastically scattered light. The total enhancement to the Raman signal observed in response to this effect (which can be as high as 10^{14} times that of the unenhanced signal) is commonly attributed to two effects: chemical and electromagnetic. The electromagnetic (EM) mechanism arises simply from the oscillations of conduction band electrons at a metal surface resulting in a larger number of scattered photons (Kneipp et al. 1999; Tao and Yang 2005). The chemical mechanism is much less well understood, but is often attributed to a charge transfer intermediate state which takes place at the strong electron coupling between the analyte and the metal surface (Zou and Dong 2006; Brus 2008). There have been many experimental demonstrations confirming that both mechanisms play key roles in SERS effects (Xu et al. 2002; Wen and Fang 2005), however it is generally believed that electromagnetic enhancement may play a greater part than chemical enhancement (Kambhampati et al. 1999; Moskovits et al. 2002; Chen and Choo 2008).

In this context, the main analytical advantages of SERS in comparison with other optical detection methods is the inherent molecular specificity which can be obtained (Kneipp et al. 1999; Qian and Nie 2008), the relatively large sensitivity, and the sharpness of the spectral signals, which can be as little as one nanometer full width at half-maximum (Ni et al. 1999). This latter advantage is to be compared to conventional fluorescent labels which average about 75 nm (Isola et al. 1998) or quantum dots which average about 30 nm (Zhang and Johnson 2006). The relative sharpness of the spectral SERS signal can facilitate multiplexing since multi-label readouts can be carried out

at single excitation wavelength (Grubisha et al. 2003) without being limited by spectral overlap.

3 Overview of traditional and advanced SERS implementations

Broadly speaking there are two ways in which a SERS detection reaction can be carried out: homogeneously, where the target becomes bound or absorbs onto the solution phase metallic nanoparticles which act as Raman enhancers, or heterogeneously, where the solution phase targets interact with the surface phase SERS active sites. The former of these has the same advantages as all homogeneous reactions (i.e. faster reaction rate and relative ease of implementation) as well as enhanced uniformity and repeatability of the SERS enhancement since the nanoparticles can be synthesized with high uniformity. Examples of such systems include the use of metal nanoshells (Jackson et al. 2003) and nanorods (Nikoobakht and El-Sayed 2003) as SERS-active substrates. One disadvantage of the homogeneous approach, however, is that because the Raman enhancers are dispersed in solution, detection sensitivity is relatively low (unless enhanced microscopy techniques like confocal are used). Over the years, numerous types of SERS-active surfaces have been demonstrated [e.g. electrochemically roughened electrodes (Liu et al. 2006), vapor-deposited metal island films (Jacobson and Rowlen 2005), periodically aligned nanoparticles (Brolo et al. 2004; Wang et al. 2005), and lithography-produced nanostructures (Dick et al. 2002) giving current researchers the ability to select the SERS substrate architecture that best matches their experimental needs (Felidj et al. 2004; Wang et al. 2005)]. While these surface phase systems can have fundamentally greater sensitivity than homogenous ones (essentially concentrating the detection zone from 3D to 2D), the analysis time can be longer (since the molecules must diffuse to the analysis site), the chip fabrication is more complicated (since nanoscopic features must be patterned) and in some cases it is difficult to obtain regular and repeatable SERS enhancement. If the enhancement is not consistent, then specific detection is still possible but reliable quantification is not. To address this, a number of scientists have recently developed unique "optofluidic" based on chip SERS devices which we will describe in greater detail in Sect. 8.

4 Application of SERS to cellular and molecular diagnostics

We begin our review of SERS based detection technology by considering those biosensing technologies that have been developed for DNA–DNA hybridizations, protein–protein interactions, and cell-based interactions. As alluded to in the introduction we have focused our attention on those technologies which have been applied to the cancer diagnostics. In the final subsection, we review a couple of advanced implementations.

4.1 Nucleic acid-based detection

To obtain SERS signals from a hybridization reaction, a Raman active molecule attached to the DNA must be brought into close contact with the metal surface. Figure 1a shows a schematic illustration of a functionalized metal nanoparticles approach in detecting DNA hybridization. In this experiment, reported by Huh et al. (2008), 50-nm diameter gold nanoparticles were chemically functionalized with the thiol modified ssDNA, making the majority of surface-bound probes accessible for hybridization. After blocking the Au metal surface with 6-mercapto-1-hexanol (MCH) to protect from non-specifically adsorbed ssDNA, the TAMRA labeled target DNA was introduced and the hybridization brought the dye into close proximity to the nanoparticle. When the sample was optically probed then the presence of a strong TAMRA Raman signature would indicate positive recognition and the absence would suggest a mismatch. Numerous variations on this approach have been developed including a "molecular beacon" based probe (MB-probe) techniques (Wabuyele and Vo-Dinh 2005; Jung et al. 2007; Vo-Dinh 2008). These approaches often use a DNA hairpin structure containing Raman active molecules and metallic nanoparticles as shown in Fig. 1b. In such systems hybridization of a complementary target sequence, which binds to the loop region, forces the dye farther away from the metallic nanoparticle reducing the strength of the emitted SERS signal. This approach offers the advantage of removing the need for a dye-labeled target sequence. Similar to the MB-probe approach, Jung et al. (2007) have shown sensitive SERS detection of DNA hybridization using the quenching of the fluorescence resonance energy transfer (FRET) signal between two fluorophores (a donor dye, TET, and an acceptor dye, TAMRA, respectively). As shown in Fig. 1c, this technique is typically based on a strong quenching of the SERS signal because the probe's hairpin structure is physically disrupted when hybridization occurs between the MB-probe and the target DNA. Another interesting recent example was presented by Fabris et al. (2007) and involved the development of a peptide nucleic acids (PNAs)-based SERS method. This unique identification scheme does not rely on the electrostatic interactions between the negatively charged DNA/DNA, rather PNAs consist of peptidomimetic neutral amide bonds. The result is faster hybridization rates due to the absence of electrostatic repulsion. Figure 1d shows a schematic illustration of the approach.

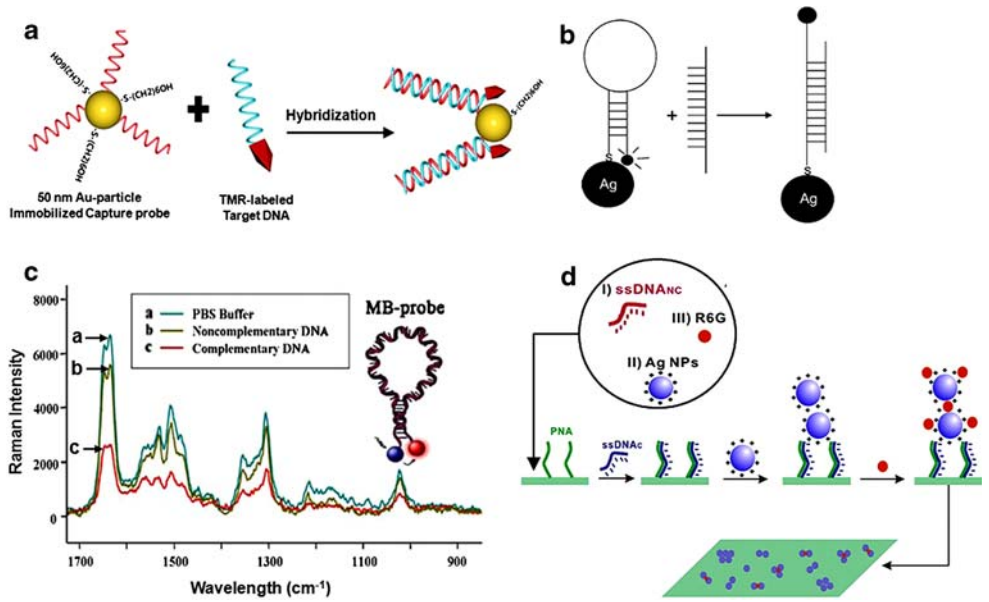

Fig. 1 **a** Schematic illustration of the DNA hybridization reaction for SERS detection (Huh et al. 2008). **b** Signaling concept of SERS molecular sentinels. SERS signal is observed when the MS probe is in the hairpin conformation (closed state), whereas in the open state the signal is diminished (Wabuyele and Vo-Dinh 2005) [Adapted with permission from the American Chemical Society]. **c** SERS spectra from the MS-probes on silver nanoparticles: *a* without the target DNA; *b* with the noncomplementary target DNA; and *c* with the complementary target DNA (Jung et al. 2007) [Adapted with permission from Springer Science and Business Media]. **d** General scheme of the peptide nucleic acids (PNAs)-based SERS assay (Fabris et al. 2007) [Adapted with permission from the American Chemical Society]. The figure shows a glass slide with surface-bound PNA. Addition of noncomplementary ssDNA ($ssDNA_{NC}$) results in no binding, leaving the original PNA surface undisturbed. When complementary ssDNA ($ssDNA_C$) is added, hybridization takes place yielding a surface with net negative charge. Addition of the positively charged Ag NPs are then attracted to the surface as shown

Several groups have combined SERS with DNA hybridization techniques for the detection of genetic markers associated with cancer (Pal et al. 2006; Culha et al. 2003). Allain and Vo-Dinh (2002) reported monitoring DNA hybridization of a fragment of the breast cancer 1 (BRCA1) breast cancer susceptibility gene on modified silver surfaces. In this experiment, when a discontinuous layer of silver is deposited onto glass slides, it was found to not only facilitate the hybridization process, but also provided the means for Raman enhancement. Sun et al. (2008) also recently reported a non-fluorescent DNA array platform on a gold-coated glass slide which is able to detect multiple DNA targets simultaneously on a single array spot. This was done using a sandwich structure utilizing DNA-Au particle (AuP)-Raman Target (RTag) probes for BRCA1 as shown in Fig. 2. The proposed approach has the potential to become a multiple detection tool for comprehensive alternative splicing profiling of BRCA1 or other genes relevant to specific cancers.

4.2 Immunoassay-based detection

Immunoassay is widely used for the detection of specific interaction between antigens and antibodies and has been adapted by several groups to enable SERS based detection. An example of this, the immunogold nanolabeling and silver staining enhancement method for SERS detection of proteins has been presented by Xu et al. (2004). Figure 3a illustrates the immunoassay system presented in this work consisting of a three layer sandwich structure. While the silver staining method can give strongly enhanced Raman signals, the technique itself is somewhat complicated due to the larger number of functionalization steps. In another example, Dou et al. (1998) proposed the use of near-infrared (NIR) SERS detection of anti-mouse IgG on Au nanoparticles regardless of binding between antibodies and antigens. Unlike the general immunoassays which require procedures for the separation of bound and free antigens, the antigen here was minimally involved in yielding a SERS signal, owing to the distancing of the Au nanoparticles from the antigen. As a result, this method enabled the direct detection of antibodies using the assignable SERS active molecules of anti-mouse IgG (such as amide groups and aromatic amino acid residues). The authors demonstrated a limit of detection (LOD) of 10^{-8} M. In a different approach, Li et al. (2008) proposed a method for SERS detection based rapid protein–protein interactions. This technique involves the specific binding of biotin-modified antibodies with protein A immobilized 30 nm gold nanoparticles and the introduction of a avidin-conjugated

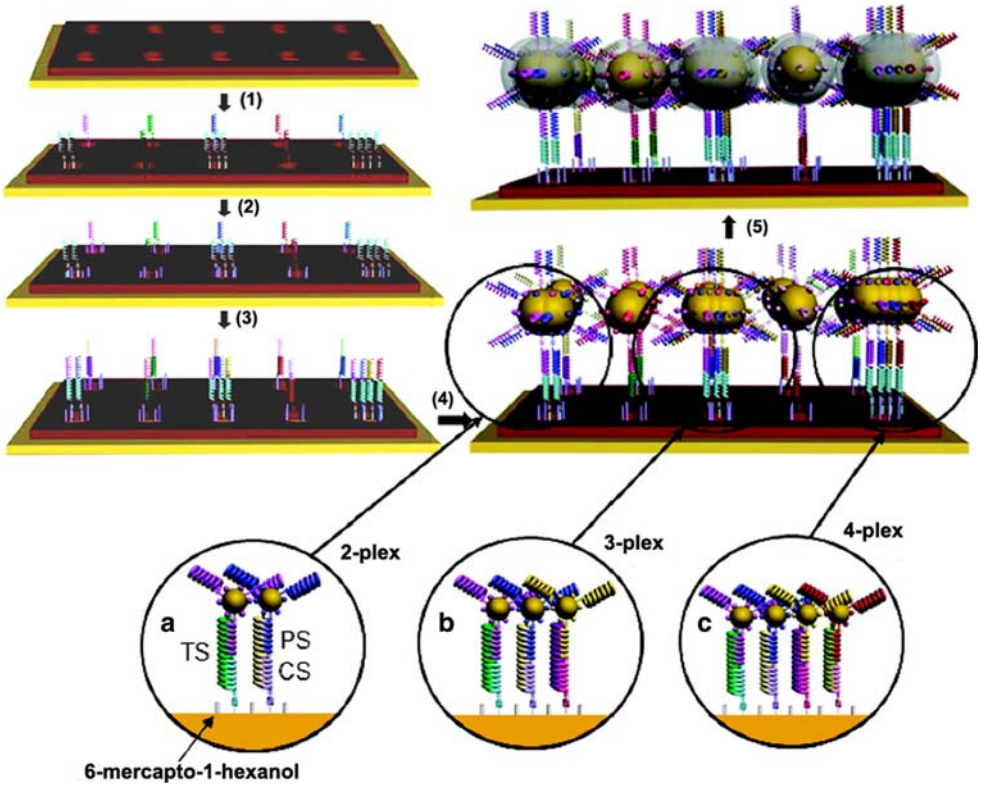

Fig. 2 DNA detection with a sandwich complex for BRCA1 alternative splice variants. *1* Immobilization of capturing strands (CS); *2* immobilization of 6-mercapto-1-hexanol to reduce nonspecific binding; *3* hybridize target strands (TS) to CS; *4* hybridize DNA-AuP-RTag probes to the overhanging region of TS; *5* silver enhancement. *a*, *b*, and *c* represent multiplex detection using DNA sequences specific to BRCA1 alternative splice variants (Sun et al. 2008) [Adapted with permission from the American Chemical Society]

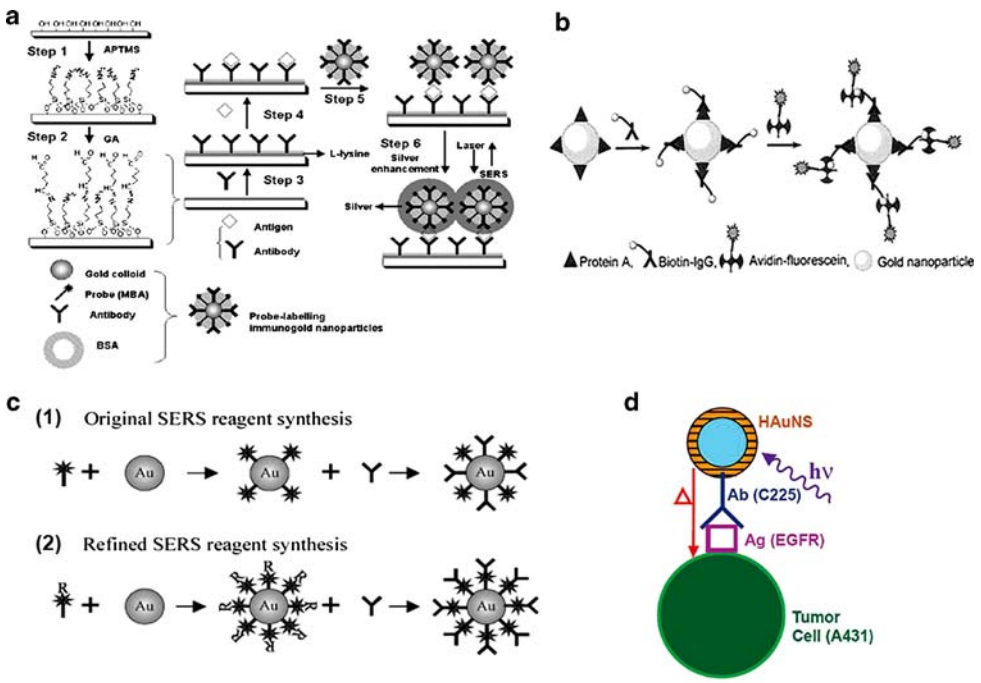

Fig. 3 **a** The process of self-assembled sandwich structure immobilized on a silicon or quartz substrate using the silver staining enhancement method (Xu et al. 2004) [Figure reproduced by permission of The Royal Society of Chemistry]. **b** Schematic representation of the SERS active system using protein–protein interaction (Li et al. 2008) [Reprinted with permission from the Japan Society for Analytical Chemistry]. **c** Dual-analyte sandwich immunoassay for identifying more subtle attributes (Grubisha et al. 2003) [Adapted with permission from the American Chemical Society]. **d** Schematic illustration of the concept of SERS detection and photothermal therapy using HGNs based on antibody (C225)–antigen (EGFR) interaction that targets A431 carcinoma cancer cells (Schwartzberg and Zhang 2008) [Adapted with permission from the American Chemical Society]

fluorescein as a Raman active dye as shown in Fig. 3b. The detection limit of IgG in a protein-A gold solution was demonstrated down to 1 ng/ml which compares with conventional technologies which typically yield between 10 ng/ml to over 3 μg/ml.

Cancer protein assay platforms using nanoparticle-based labels (Yakes et al. 2008) have also been developed. These methods relies on the construction of gold nanoparticles modified with both an inherently strong Raman enhancer and an antibody, referred to as extrinsic Raman labels (ERLs). This ERL approach allows the acquisition of a strong signal enhancement from a common reporter coated on nanometer-sized metal nanoparticles (Fig. 3c). One of the early applications illustrating the potential utility of ERLs in early disease diagnosis was the detection of prostate specific antigen (PSA) (Grubisha et al. 2003).

4.3 Cell-based detection and other studies

Living- or whole-cell Raman spectroscopy can serve as the basis for reliable identification of molecular events inside intact cells (Jarvis and Goodacre 2008). Breuzard et al. (2004) performed living cell studies using SERS spectroscopy and proposed that it could be an effective method in studying the process of the anticancer drug mitoxantrone (MTX) absorption into the plasma membrane of living cells. Shamsaie et al. (2007) demonstrated a cellular SERS probe they called intracellular grown Au nanoparticles (IGAuNs) using nanoparticles that grow inside MCF10 epithelial cells. Since large nanoparticles are not able to pass through the nuclear membrane pores, they cannot reach the cytoplasm or nucleus. They also show poor "controllability" when inserted inside the cell (Chithrani et al. 2006; Shamsaie et al. 2007). The presented IGAuNs, however, can be precisely controlled to overcome some of the inherent drawbacks, and act as a potential SERS substrate to understand intracellular events. Figure 3d shows an example of an interesting approach to bioconjugated nanoparticle techniques using hollow gold nanoparticles (HGN) based on antibody–antigen interaction. This approach was shown to exhibit good potential for both SERS detection and thermal therapy of cancer since the metal nanostructures are also excellent photothermal converters upon absorbing light at particular wavelength (Schwartzberg and Zhang 2008).

4.4 Tip-enhanced Raman spectroscopy (TERS) and surface enhanced Raman imaging (SERI)

Though nanoparticle approaches provide great localizability of the SERS signal, it can be difficult to spatially localize the particles themselves (ensuring the desired location is probed). Recently, there has been great progress towards solving this problem through the development of tip-enhanced Raman spectroscopy or TERS (Bruno Pettinger 2002; Pettinger et al. 2004). The concept of TERS was first presented by Wessel (1985), and developed independently in 2000 by several groups (Verville and Sanderson 2000; Hayazawa et al. 2001; Pettinger et al. 2004). A typical experimental implementation involves coupling a scanning tunneling microscope (STM) (or an atomic force microscope, AFM) with Raman spectroscopy. As with the structures discussed above, a large electromagnetic near-field enhancement arises when the metal tip (which is often much less than 100 nm, recently <20 nm radius) in these instruments is illuminated (Sun and Shen 2003; Demming et al. 2005; Notingher and Elfick 2005). In addition to localization, TERS allows vibrational spectra to be obtained from molecules adsorbed onto any number of different substrates (i.e. they no longer have to be made of structured metal) allowing for a much broader range of samples to be probed. Figure 4 shows the illustration of the working principle of TERS and the application of

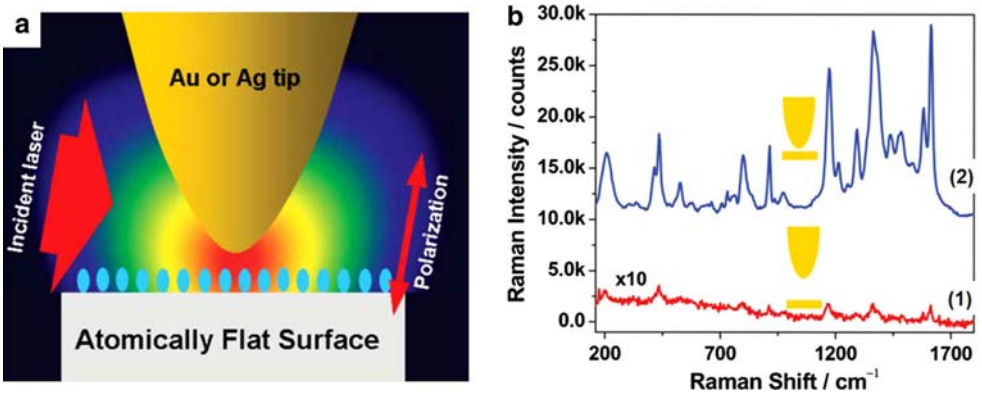

Fig. 4 **a** A schematic illustration of the working principle of tip-enhanced Raman spectroscopy: light with appropriate wavelength and polarization is illuminated at the gap between the tip and the surface. **b** SERS spectra of malachite green isothiocyanate (MGITC) before (*1*) and after (*2*) the approaching of the Au tip (Tian et al. 2007) [Reproduced by permission of The Royal Society of Chemistry]

Malachite Green Isothiocyanate (MGITC) SERS spectra before and after the approaching of the Au tip (Tian et al. 2007).

Conventional Raman imaging enables one to capture the spatial distribution of the Raman spectra from which molecular composition and configuration can be obtained (Shafer-Peltier et al. 2002; Sweder W. E. van de Poll 2002). As an example of the implementation of this technique for cancer diagnostics, Shafer-Peltier et al. (2002) recently explored in situ diagnosis of breast cancer during needle biopsy via an optical fiber probe. In general, however, long integration times were required due to the weak intrinsic Raman signal. This greatly hinders the technique's application to live cell imaging application due to the possibility of photothermal cell damage. Surface enhanced Raman imaging or SERI, on the other hand, allows the acquisition of a much stronger signal reception and provides shorter integration times required for living cell imaging applications. In a recent work, Wabuyele et al. (2005) developed a confocal surface-enhanced Raman imaging (SERI) system that combines hyper-spectral imaging capabilities with SERS to identify labeled silver nanoparticles in cellular compartments with high spatial and temporal resolution. The approach allows the recording of the entire SERS signal from every pixel in the field of view. The resulting hyper-spectral image is presented as a three-dimensional (3D) data cube, consisting of two spatial dimensions (x, y) defining the image area of interest and the spectral dimension (λ). This spectral imaging technique is used to chemically identify the material at each pixel. This technique offers a significant application for molecular signaling and monitoring of other nanoscale bimolecular phenomena (Yu et al. 2007). Qian et al. (2008) applied this type of approach to in vivo cancer imaging using pegylated gold nanoparticles.

5 Single molecule analysis using SERS

The extremely large SERS cross sections (roughly the 10^{14} enhancement discussed earlier) for near-infrared excitation of molecules attached to colloidal silver or gold clusters (Kneipp et al. 1998a, 1999; Michaels et al. 1999; Haslett et al. 2000) makes it possible to use SERS to provide molecularly specific information on a very small number of molecules. Near infrared (NIR) excitation also offers the advantage of decreasing the fluorescence background which interferes with traditional single-molecule Raman detection (Kneipp et al. 1999). This has made SERS of great practical interest to both the nanomaterials and single-molecule spectroscopy communities (Qian and Nie 2008). An extension of the standard SERS approach particularly relevant to single molecule spectroscopy is surface enhanced resonance-Raman scattering (SERRS). SERRS offers sharper and specific fingerprint signals under the suitable laser excitation frequency than traditional SERS and thus, produces more reliable, sensitive, and reproducible spectra (Mahajan et al. 2007; Faulds et al. 2008). Single molecule analysis using SERRS was recently reported by Nie and Emory (1997) using 514 nm excitation and Rhodamine 6G adsorbed on silver nanoparticles. Single molecule detection was done by estimating the number of molecules in the focal area and observing strong signal fluctuations and spectral changes that occurred on the time scale of seconds. Ultimately, one of the most promising applications of single-molecule SERS could be in the field of rapid detection and identification of individual DNA bases using the Raman structurally specific spectroscopic characterization of individual base pairs in DNA fragments without the use of fluorescent or radioactive labels (Kneipp et al. 1998b). Such systems may eventually have significant impact on cancer diagnostics by enabling rapid genetic screening or label free detection of dilute low molecular weight biomarkers.

6 Technologies for multiplexed SERS detection

For high-throughput applications, it is often desirable to use a detection method which enables one to simultaneously screen for a large number of different biological targets in a given sample (Sun et al. 2008). Since the highly compound specific Raman spectral bands minimize spectral overlap of different labels, the potential to spectrally analyze multi-component samples or to use multiple labels simultaneously is one of the primary advantages of SERS over fluorescence-based strategies (Allain and Vo-Dinh 2002; Vo-Dinh et al. 2002). A demonstration of this is the detection of specific target DNA sequences using a novel "molecular sentinel (MS)", analogous to the molecular beacon technology discussed in Sect. 4.1, (Pal et al. 2006; Wabuyele and Vo-Dinh 2005; Vo-Dinh 2008). Since the detected emission is the SERS spectra (which is easier to differentiate from dye-to-dye than the florescence spectra) a larger number of targets can be screened for without the fear of ambiguous results. An example of multiplxed DNA detection applied to Cancer screening was the surface-enhanced Raman gene (SERGen) probes reported by Vo-Dinh et al. (2002) which were used to detect DNA targets via hybridization onto the nano structured metallic substrates. In this approach, upon binding of the individual dyes to BRCA1 oligonucleotides, the SERS active dye labeled probes were introduced for the detection of their complementary sequences via hybridization, followed by the SERS based detection of the hybridized probe. As an alternative to the traditional DNA array format approach, Cao et al. (2002) reported the SERS multiplexed DNA

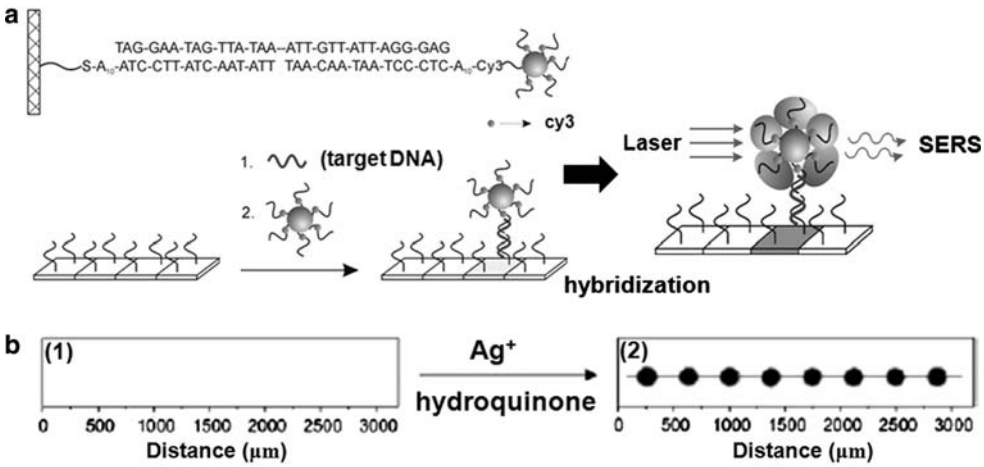

Fig. 5 **a** Nanoparticle probes for multiplexed detection of oligonucleotide targets. **b** Flatbed scanner images of microarrays hybridized with nanoparticles (*1*) before and (*2*) after Ag enhancing (Cao et al. 2002). Reprinted with permission from American Association for the Advancement of Science (AAAS)

detection with gold nanoparticle probes conjugated oligonucleotides and Raman-active dyes (see Fig. 5). To achieve a more sensitive SERS signal, the gold nanoparticles are coated with silver which acts as a SERS promoter. After Ag enhancing, the particle can grow around the SERS active dye-labeled gold nanoparticle probes, leading to strongly Raman scattering enhancement. In this experiment, six different DNA targets were analyzed using six SERS active dye-labeled probes and showed the LOD of 20 fM.

Multiplexed SERS immunoassay techniques have also been developed for protein marker screening. For example, Jun et al. (2007) have proposed SERS-encoded polystyrene (PS) beads containing Raman active small compound labeled silver nanoparticles with a silica shell, enabling them to obtain reproducible spectra for multiple samples. As another example, Grubisha et al. (2003) have also demonstrated the detection of prostate specific antigen using a sandwich immunoassay based on SERS. This interesting approach achieved the detection limits of 1 pg/ml target cancer protein in human serum using the directed uptake of gold nanoparticles labeled with both tracer antibodies and strong Raman reporter molecules.

7 In vivo and in situ applications of SERS and Raman Spectroscopy

SERS based in vivo diagnostics is much more complicated than in vitro assays due to the complexities in optically accessing the samples and worries about nanoparticle toxicity. One approach to minimizing the toxicity of metal nanoparticles is to cover them with a nontoxic coating (e.g. silica) or other biocompatible materials. For example Kim et al. (2006) demonstrated the biocompatible, photostable, and multiplexing-compatible SERS active tagging material using the silver nanoparticle-embedded silica spheres which incorporate a variety of organic Raman label compounds for cellular cancer targeting in living cells. In an interesting recent example, Qian et al. (2008) has reported biocompatible and nontoxic nanoparticles for SERS based in vivo tumor targeting and detection based on pegylated gold nanoparticles. In that work targeted gold nanoparticles are prepared by using a mixture of thiol-PEG and a heterofunctional PEG (SH–PEG–COOH). The epidermal growth factor receptor (EGFR) (Paez et al. 2004) was covalently conjugated at the exposed end of the heterofunctional PEG (SH–PEG–COOH) with high specificity and affinity. The results of this work demonstrated that highly sensitive SERS signals can be obtained from EGFR-positive cancer cells of subcutaneous and deep muscular samples. In another approach, Shim et al. (2000) reported the first in vivo Raman spectra of human gastrointestinal (GI) tissues measured during routine clinical endoscopy. This was achieved by using a NIR fiber-optic Raman probe that was passed through the endoscope instrument channel and placed in contact with the tissue surface. This demonstrated the possibility of obtaining in vivo Raman spectra from various GI tract organs, with acceptable signal strength and short collection times. This step is required to determine the diagnostic accuracy of the technique before initiating systematic clinical trials (Wolfbeis 1991; Vo-Dinh et al. 2002; Utzinger and Richards-Kortum 2003). Recent work by Tang et al. (2007) reported the ability of NIR-SERS enhanced by gold nanoparticles to obtain detailed chemical structural information with high resolution inside a single cancer cell. The cancer cell specimens allow the specific SERS characterization of intracellular chromophores, because gold nanoparticles and Raman dyes are able to enter the living cell and reside in the cytoplasm and around the nucleus.

These bioconjugated SERS approaches could also contribute to the biomolecular analysis of cancer tissue specimens removed by surgery and for circulating tumor

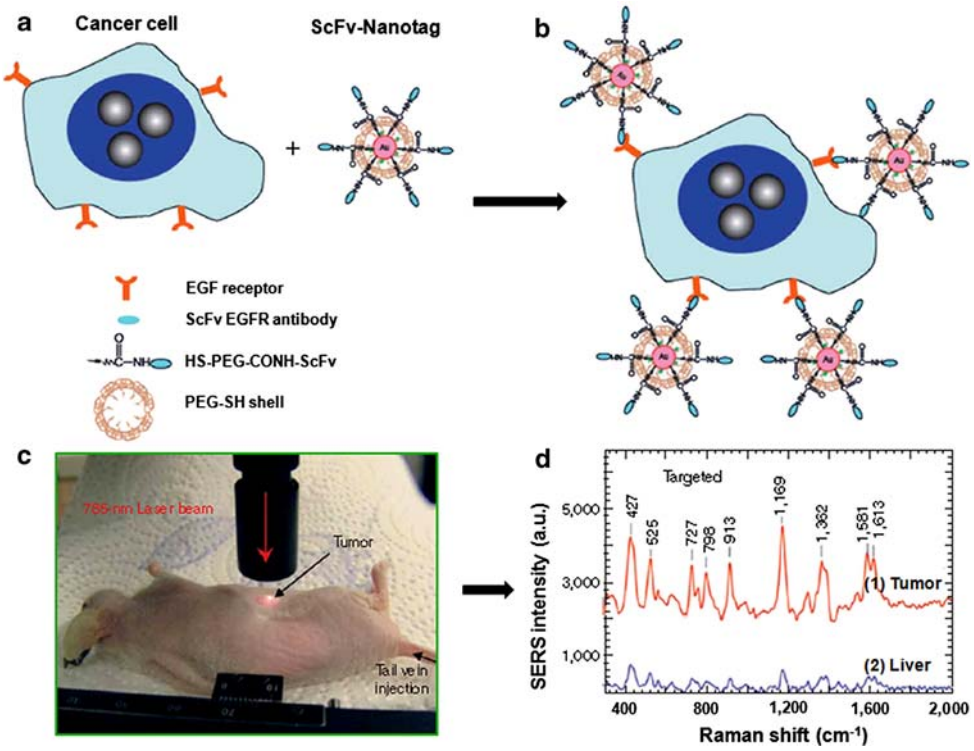

Fig. 6 Cancer cell targeting and spectroscopic detection by using antibody-conjugated SERS nanoparticles. **a**, **b** Preparation of targeted SERS nanoparticles by using a mixture of SH–PEG and a hetero-functional PEG (SH–PEG–COOH). Covalent conjugation of an EGFR-antibody fragment occurs at the exposed terminal of the hetero-functional PEG. **c** Photographs showing a laser beam focusing on the tumor. **d** In vivo cancer targeting and SERS detection by using ScFv antibody conjugated gold nanoparticles that recognize the tumor biomarker EGFR. In vivo SERS spectra were obtained from the tumor site (*1*) and the liver site (*2*) with 2-s signal integration and at 785 nm excitation (Qian et al. 2008). Figure adapted by permission from Macmillan Publishers Ltd: Nature Biotechnology

cells captured from peripheral blood samples (Nie and Emory 1997; Qian and Nie 2008). For in vivo tumor SERS detection, Qian and Nie (2008) used an infrared dye (diethylthiatricarbocyanine, DTTC) as a spectroscopic reporter, and achieved surface-enhanced resonance Raman scattering (SERRS) at 785-nm excitation (Fig. 6). The technique provided a rapid and non-destructive analytical capability allowing for structural fingerprinting because the chromophores adsorbed on SERS active surfaces were protected from photo-degradation by efficient energy transfer to the metal particle. Figure 6 shows the schematic diagram of cancer cell targeting procedures and the results of in vivo SERS detection by using antibody-conjugated SERS nanoparticles.

8 Optofluidic-based SERS technologies

Recently a number of new optofluidic approaches, which aim to integrate directly SERS detection systems with micro- and nano-fluidics, have been developed. These systems offer several advantages including: small sample requirements, fast mixing, and improved analytical performance (Docherty et al. 2004; Chen and Choo 2008). In our group, a novel microfluidic technique for on-chip SERS based biomolecular detection, exploiting the use of electrokinetically active microwells has been recently reported (see Fig. 7a, b) (Huh et al. 2008). This approach combines the advantages of existing homogeneous (solution phase) and heterogeneous (surface phase) on-chip techniques by enabling active mixing to enhance the rate of binding between the SERS enhancers and the biomolecular targets as well as rapid concentration of the product for surface phase optical interrogation. Cheng et al. (2007) have recently presented an integrated AC dielectrophoretic (DEP) microfluidic platform that can filter, focus, sort, and trap heterogeneous bioparticle, and identify two gastrointestinal bacteria, *E. coli Nissle* and *Lactobacillus*, by SERS on a single chip. This platform could provide a significant contribution to the ongoing efforts to miniaturize multi-target pathogen detection. As another example, Park et al. (2005) described the use of an alligator-teeth-shaped PDMS microchannel to promote mixing between the target analyte and the metallic colloids used as SERS enhancers. This helps to address the problem of the slow reaction time and low reproducibility caused by the diffusion-limited kinetics in the solution phase. With optimized mixing, this chip-based approach can reduce the reaction/detection time to a few minutes. Strehle et al. (2007) showed the application of a liquid/liquid microsegmented flow for serial high-throughput microanalytical systems on the model compound crystal violet. This setup could overcome the well-known problem of adhesion of colloid/analyte conjugates to the optical windows of detection cuvettes, which causes the so-called "memory effect" reducing the limit of detection for such systems. Wang et al. (2007) presented a nanofluidic trapping device that concentrated SERS nanoparticles at the inlet to a bottleneck shape nanochannel greatly enhancing the Raman

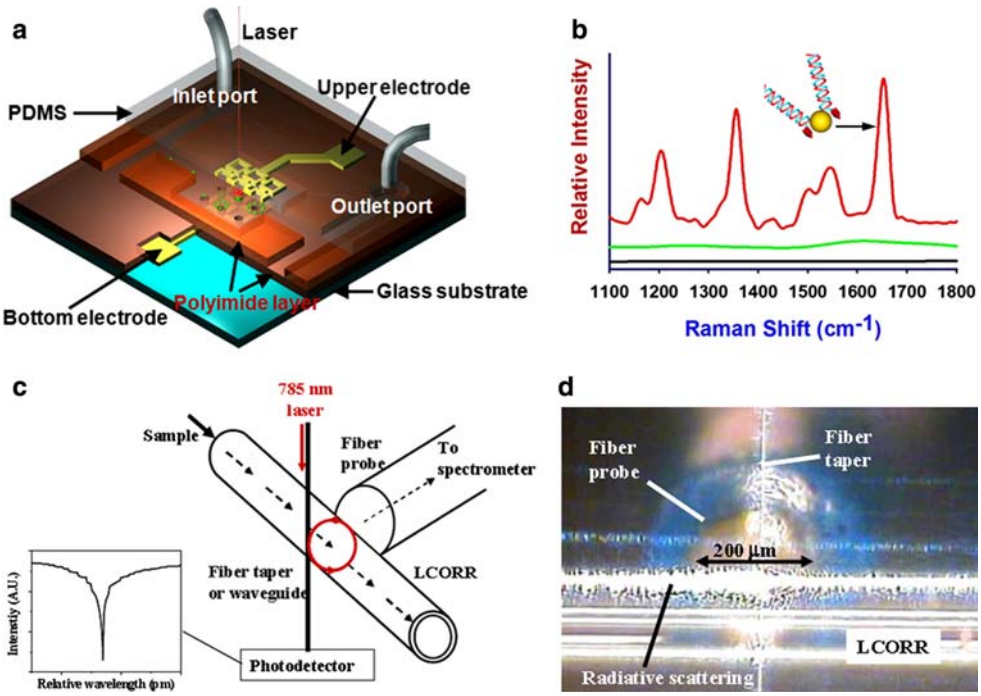

Fig. 7 **a** Schematic representation of the electrokinetically active microwells for enhanced SERS detection (Huh et al. 2008). **b** SERS spectra of the DENV-2a hybridization reaction procedures. (1) SERS spectra of gold NPs after immobilization of capture probes and application of MCH to protect against non-specific absorption. (2) SERS spectra after hybridization with DENV-4a (negative control) and (3) with DENV-2a (target DNA) using the functionalized gold NPs. **c** Experimental setup for measuring the Raman scattering signal from the LCORR. **d** Snapshot showing the LCORR capillary, the fiber taper, and the fiber probe (White et al. 2007) [parts **c** and **d**, reprinted with permission of the Optical Society of America]

signal. Another approach by Abu-Hatab et al. (2007) used demonstrated the detection of a number of analytes down to the nM and sub-pM range by detecting the major SERS bands. White et al. (2007) recently introduced an alternative approach involving SERS detection using integrated optical waveguides (see Fig. 7c, d). In this and similar systems, light is confined to travel within the high refractive index core of the waveguide but a portion of it (known as the evanescent field) extends a few hundred nanometers outside the waveguide, into the surrounding environment. This allows it to interact with molecules which are either bound or drift into the near field. This optical sensor technique has several advantages over the other optical sensors which are usually stated as a compactness, very high sensitivity and possibility of mass production.

9 Summary and conclusions

In this paper we have reviewed SERS optical sensor technology with an application focus on cancer diagnostics. Particular attention has been paid to the sensitivity, specificity and multiplexing capabilities of each technique as a gauge as to how well it is likely to meet future needs in a variety of fields ranging from in vivo/in vitro medical diagnostics, pharmaceutical discovery and cancer detection. In addition to sensitivity enhancement, the advantages of SERS in comparison with other optical diagnostic techniques include the ability to obtain molecularly specific information about the probed target (unlike SPR which can only monitor changes in accumulated mass) and relatively narrow spectral bandwidths (enabling greater multiplexibility than florescence techniques). Disadvantages include the relative complexity of the output signal (particularly for multicomponent systems) and the difficulty in obtaining repeatable signal enhancements. To address these disadvantages novel approaches are required to: better control the gap distance between metallic enhancer and reporter, eliminate the SERS background signal by blocking of non-specific binding and, help differentiate a greater number of SERS active tags in a complex solution.

As we have attempted to focus on here, recent advances in instrumentation and experimental design have led to the increasing application of SERS to the molecular diagnosis of cancer (by monitoring DNA–DNA hybridizations, protein–protein interactions, and other ligand–receptor interactions) and the study of cancer cells under in vivo and in situ conditions. An important advancement related to the latter of these has been the development of biocompatible, non-toxic, nanoparticle based SERS active tags. These types of tags and others which address toxicity concerns will certainly play an even more important role in next generation methods of SERS based in vivo cancer diagnostics. For nearer term in vitro diagnostics, the integration of optical detection technologies with microfluidic systems into "optofluidic" devices represents an emerging area. In addition to the standard benefits of microfluidics, one of the biggest advantages of this approach is likely to be the greater ability to obtain reproducible SERS enhancement factors through formation of consistent SERS-active clusters or regular nano-patterning of surfaces resulting in more reliable quantitative analysis. As with many other

microfluidic applications, current devices are somewhat limited since off-chip detection systems (e.g. raman microscopes) and sample preparations are carried out off-chip. By incorporating advancements in other areas of optofluidics and microfluidics, next generation devices are likely to integrate all these elements into a single system.

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